

## WHAT IS CLAIMED IS:

1. A fluorescence detection method in which a sample solution containing fluorescence molecules is in contact with a substrate, light is entered at an incident angle within the range of allowing total reflection at the interface between the substrate and the sample solution, and fluorescence generated from the fluorescence molecules excited by an evanescent field generated in a vicinity of the interface is detected,
  - 5 wherein the substrate has a laminated structure of a dielectric base material layer, a metallic thin film layer and a dielectric coating layer so that the dielectric coating layer contacts the sample solution, and
  - 10 the light is entered at a specific incident angle which enables excitation of surface plasmon resonance at the interface between the metallic thin film layer and the dielectric coating layer.
- 15 2. A fluorescence detection device comprising:
  - a substrate which comes into contact with a sample solution containing fluorescence molecules and has a laminated structure of a dielectric base material layer, a metallic thin film layer and a dielectric coating layer in which the dielectric coating layer contacts the sample solution,
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  - illumination means for entering light at an incident angle within the range of allowing total reflection at the interface between the substrate and the sample solution, which is set to make the light enter at a specific incident angle which enables excitation of surface plasmon resonance at the interface between the metallic thin film layer and the dielectric coating layer, and
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  - detection means for detecting fluorescence generated from the fluorescence molecules excited by an evanescent field generated in a vicinity of the interface.
- 30 3. The fluorescence detection device according to claim 2, wherein the metallic thin film is made of a material with high conductivity.

4. The fluorescence detection device according to claim 3, wherein the material with high conductivity is silver.
5. The fluorescence detection device according to claim 2, wherein the thickness of the dielectric coating layer is as large as a wavelength of light entering from the illumination means so that multiple reflection occurs within the dielectric coating layer.
- 10 6. The fluorescence detection device according to claim 2, wherein the illumination means comprises a semiconductor laser as an excitation light source.
7. The fluorescence detection device according to claim 2, wherein the illumination means comprises a light emitting diode as the excitation light source.
- 15 8. The fluorescence detection device according to claim 2, wherein the detection means is a CCD.
9. The fluorescence detection device according to claim 2, wherein the detection means is a photodiode.
- 20 10. A total internal reflection fluorescence microscope formed of the fluorescence detection device according to any one of claims 2 to 4.
- 25 11. A biomolecule detection device formed of the fluorescence detection device according to any one of claims 2 to 4.